CHAPTER III

RESULTS

1. Analysis of exsheathment and midgut invasion of *B. malayi* microfilariae in *Ae. aegypti* using LM

Mosquitoes were infected by blood feeding and at various time points PIBM dissected, microfilariae with intact morphology were counted and the presence/absence of the sheath recorded (Figure 3.1). Table 3.1 shows the average number and percentage of nocturnally sub-periodic *B. malayi* microfilariae in the midgut and hemolymph of *Ae. aegypti* at different time points PIBM ranging between 5 min and 96 h. The average total number of microfilariae found in mosquitoes varied from approximately 34 to 48 per mosquito up until 36 h PIBM, but with no obvious trend in numbers over this time period. The highest number of microfilariae recorded in a single mosquito up to 36 h PIBM was 117, the lowest was 12. However, from 48 h PIBM onwards there was a clear decrease in numbers, the average number of microfilariae found decreased more than two fold to 48 h, and further decreased at 72 h and 96 h PIBM. In addition to changes in the total number of microfilariae per mosquito PIBM, their distribution also changed as some microfilariae crossed the midgut wall and were found in the hemolymph. The earliest time point microfilariae were found in hemolymph was after only 2 h, the highest numbers were recorded at 24-48 h, but at all points PIBM these only represented small numbers of microfilariae. All the microfilariae used in experiments were sheathed at the beginning, and

accordingly at 5 min PIBM (the first sampling point) almost all had still retained their sheath, and exsheathed microfilariae in the midguts were less than 1% of the total at this time point. However, at later time points PIBM the average percentage of exsheathed microfilariae found in midguts progressively increased to about 20%, 60%, 80%, 90%, and 100% at 1 h, 2-5 h, 6-12 h, 18-36 h, and 48 h PIBM, respectively. Microfilariae were observed in the mosquito hemolymph between 2 h and 96 h PIBM, and importantly all of these microfilariae found in the hemolymph were exsheathed. These results indicate that the microfilariae are unable to retain their sheath until they reach the hemocoel in this refractory mosquito, and appear to lose their sheaths in the midgut prior to or in the act of crossing the midgut wall.

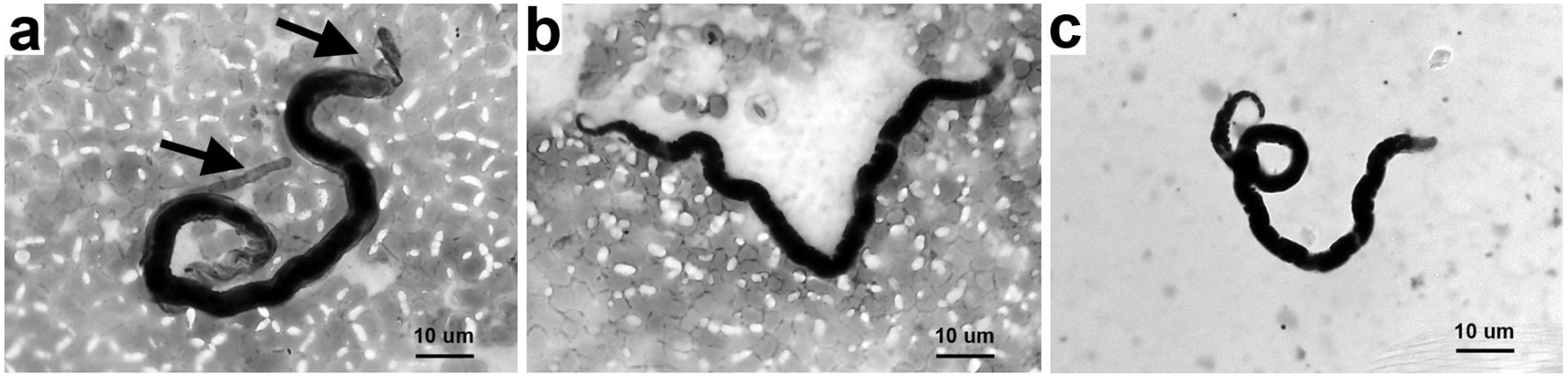

Figure 3.1 Representative images of *B. malayi* microfilariae with intact morphology in *Ae. aegypti*. (a) A sheathed microfilaria in the midgut. Arrows indicate the microfilarial sheath. (b) An exsheathed microfilaria in the midgut. (c) An exsheathed microfilaria in the hemolymph.

Table 3.1 Average number and percentage of microfilariae (mff) of NSP *B. malayi* in the midgut and hemolymph in *Ae. aegypti* at different times PIBM based on 30 infected mosquitoes per time point from triplicate experiments (n=10/mosquito/time point/experiment; 450 total)

Time PIBM	Average No. of total mff found per mosquito (range)	Average No. of mff found in midgut per mosquito (range)	Average No. of mff found in hemolymph per mosquito (range)	Mff found in midgut per mosquito		Mff found in hemolymph per mosquito	
				Average No. of sheathed mff (%)	Average No. of exsheathed mff (%)	Average No. of sheathed mff (%)	Average No. of exsheathed mff (%)
5 min	34.33 (12-60)	34.33 (12-60)	0	34 (99.04)	0.33 (0.96)	0 (0)	0 (0)
1h	38.87 (20-66)	38.87 (20-66)	0	30.13 (77.51)	8.73 (22.49)	0 (0)	0 (0)
2h	46.40 (25-76)	45.53 (24-76)	0.87 (0-2)	18.07 (39.69)	27.47 (60.31)	0 (0)	0.87 (100)
3h	42.33 (24-67)	40.73 (24-65)	1.60 (0-4)	15.53 (38.13)	25.20 (61.87)	0 (0)	1.60 (100)
4h	43.93 (18-67)	41.20 (18-63)	2.73 (0-5)	16.07 (39.00)	25.13 (61.00)	0 (0)	2.73 (100)
5h	38.93 (27-55)	35.80 (25-52)	3.13 (1-7)	14.33 (40.03)	21.47 (59.97)	0 (0)	3.13 (100)
6h	42.40 (19-83)	39.47 (19-77)	2.93 (0-6)	9.13 (23.13)	30.33 (76.87)	0 (0)	2.93 (100)
12h	42.73 (21-71)	38.13 (18-67)	4.60 (2-9)	8.00 (20.98)	30.13 (79.02)	0 (0)	4.60 (100)
18h	46.00 (22-63)	39.00 (11-60)	7.00 (3-11)	4.00 (10.27)	35.00 (89.73)	0 (0)	7.00 (100)
24h	48.13 (26-117)	36.87 (20-102)	11.27 (4-22)	3.33 (9.03)	33.53 (90.97)	0 (0)	11.27 (100)
30h	36.27 (18-61)	28.87 (10-57)	7.40 (3-11)	2.80 (9.70)	26.07 (90.30)	0 (0)	7.40 (100)
36h	34.20 (15-49)	23.20 (9-36)	11.00 (6-20)	1.80 (7.76)	21.40 (92.24)	0 (0)	11.00 (100)
48h	17.27 (10-28)	11.13 (7-17)	6.13 (1-11)	0 (0)	11.13 (100)	0 (0)	6.13 (100)
72h	5.53 (2-10)	0	5.53 (2-10)	0 (0)	0 (0)	0 (0)	5.53 (100)
96h	2.60 (1-6)	0	2.60 (1-6)	0 (0)	0 (0)	0 (0)	2.60 (100)

2. Analysis of exsheathment and midgut invasion of *B. malayi* microfilariae in *Ae. aegypti* using SEM

To investigate the exsheathment of the microfilariae in the midguts and their penetration across the *Ae. aegypti* in greater detail, SEM was used. SEM analysis at early time points revealed that some sheathed microfilariae were surrounded by small granular particles, suggestive of some kind of degradative process (Figure 3.2a). Breaking up or maceration of the microfilarial sheath was also observed in the midguts of the mosquitoes dissected at 3 h PIBM (Figures 3.2b-d). Between 5 min and 4 h PIBM, exsheathed microfilariae were also observed inside the midgut lumen of mosquitoes, either in contact with or close to the PM (Figures 3.2e, f). Invasion of the *B. malayi* microfilariae into the hemocoel was observed in SEM samples taken 2-4 h PIBM. The microfilariae could be seen to have penetrated the internal face of the PM by their anterior part, and then the midgut epithelium before entering the hemocoel (Figure 3.3). There was no evidence that the PM functioned as a barrier to the migration of the microfilariae out of the blood meal at any times PIBM that they were examined. All *B. malayi* microfilariae seen penetrating the midgut epithelium were seen to be exsheathed in SEM (Figure 3.3), confirming the results from LM observations. In contrast, Figures 3.4a-f demonstrate the presence of sheathed microfilariae in the lumens of the midguts examined during 1-36 h PIBM. Melanization of some microfilariae were observed in the hemocoel at 96 h PIBM (Figure 3.5). No mature L3 larval stages were observed in the thoracic muscles or elsewhere in mosquitoes examined at 96 h PIBM.

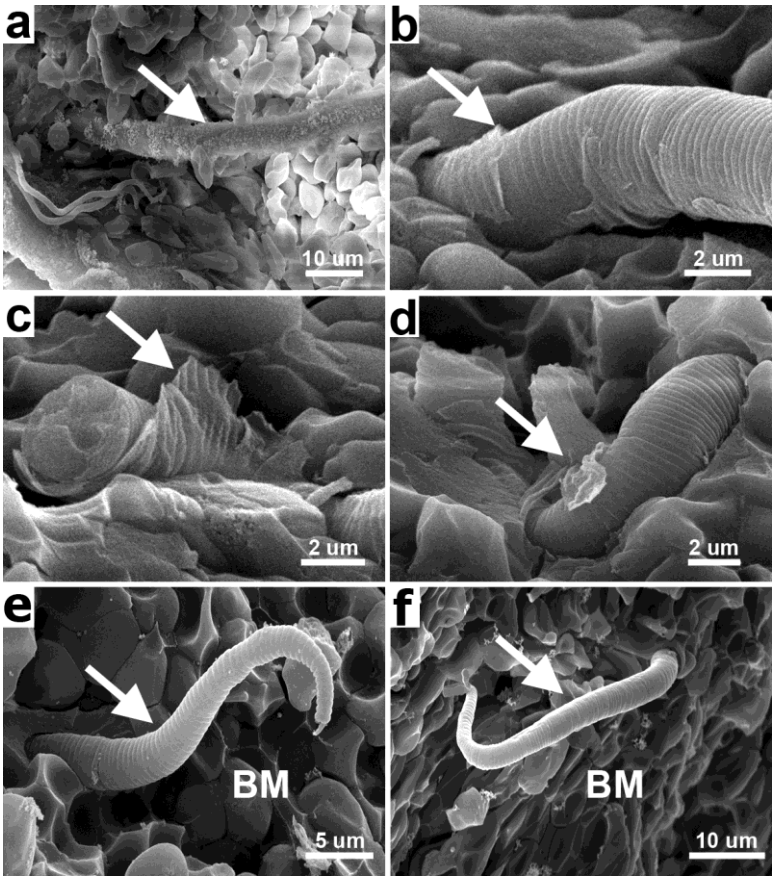

Figure 3.2 SEM micrographs of abdominal midguts showing the exsheathment of microfilariae. (a) A representative image of a sheathed microfilariae (arrow) surrounded by small particles in the midgut lumen observed in a midgut dissected at 1 h PIBM. (b-d) Representative examples of maceration of the sheaths (arrows) of microfilariae in midguts dissected at 3 h PIBM. (e-f) Representative examples of exsheathed microfilariae (arrows) inside the blood meal (*BM*) in the midgut dissected at 3 h PIBM.

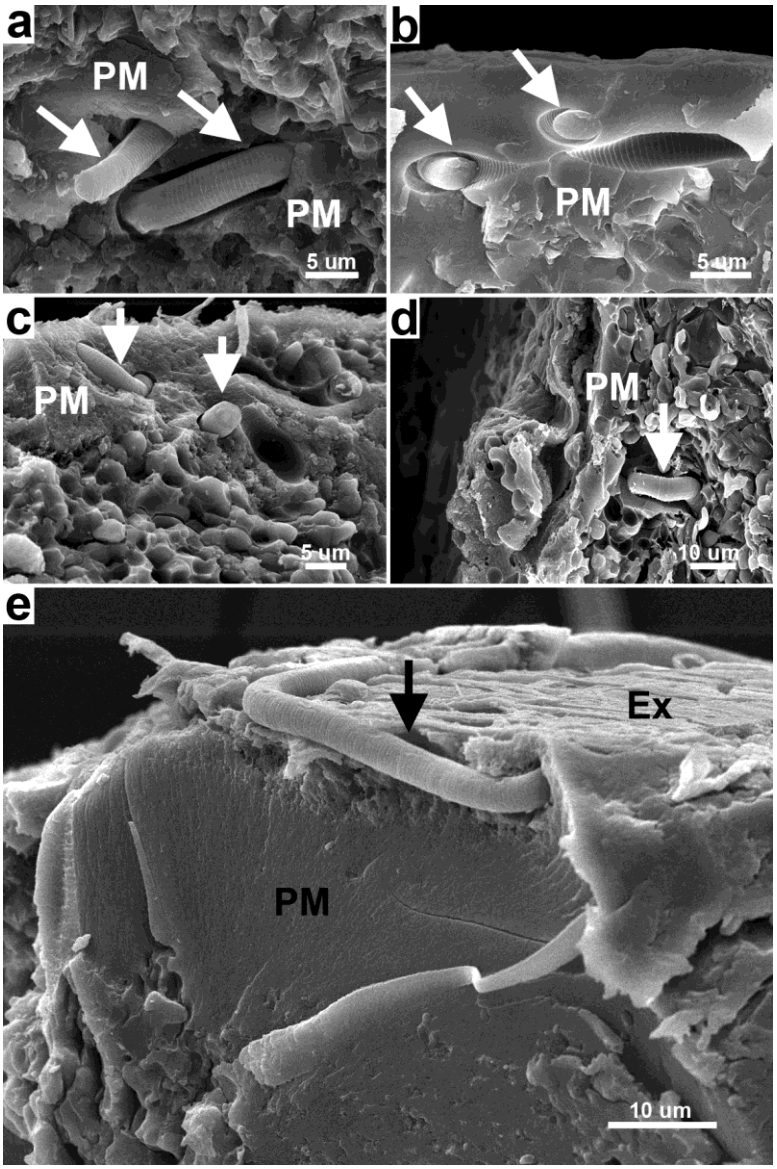

Figure 3.3 SEM micrographs of fractured abdominal midguts showing the invasion process of microfilariae. Specimens are from the midgut lumen and hemocoel at 4 h PIBM. (a,b) Exsheathed microfilariae (arrows) close to the PM. (c) Exsheathed microfilariae (arrows) crossing the PM. (d) Fractured region showing exsheathed microfilariae (arrow) during invasion into the PM. (e) An exsheathed microfilaria (arrow) penetrating across the PM and epithelium into the hemocoel and lying on the external surface (*Ex*) of the midgut.

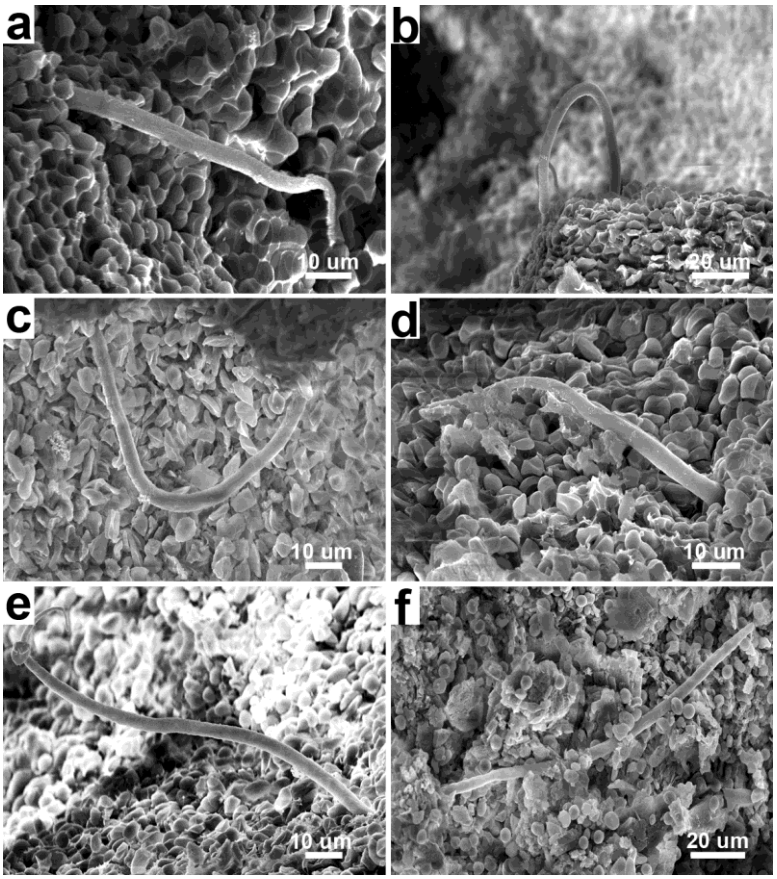

Figure 3.4 SEM micrographs showing sheathed microfilariae in the midgut lumen. (a-f) Representative examples from midguts dissected at 1 h, 3 h, 12 h, 24 h, and 36 h PIBM, respectively.

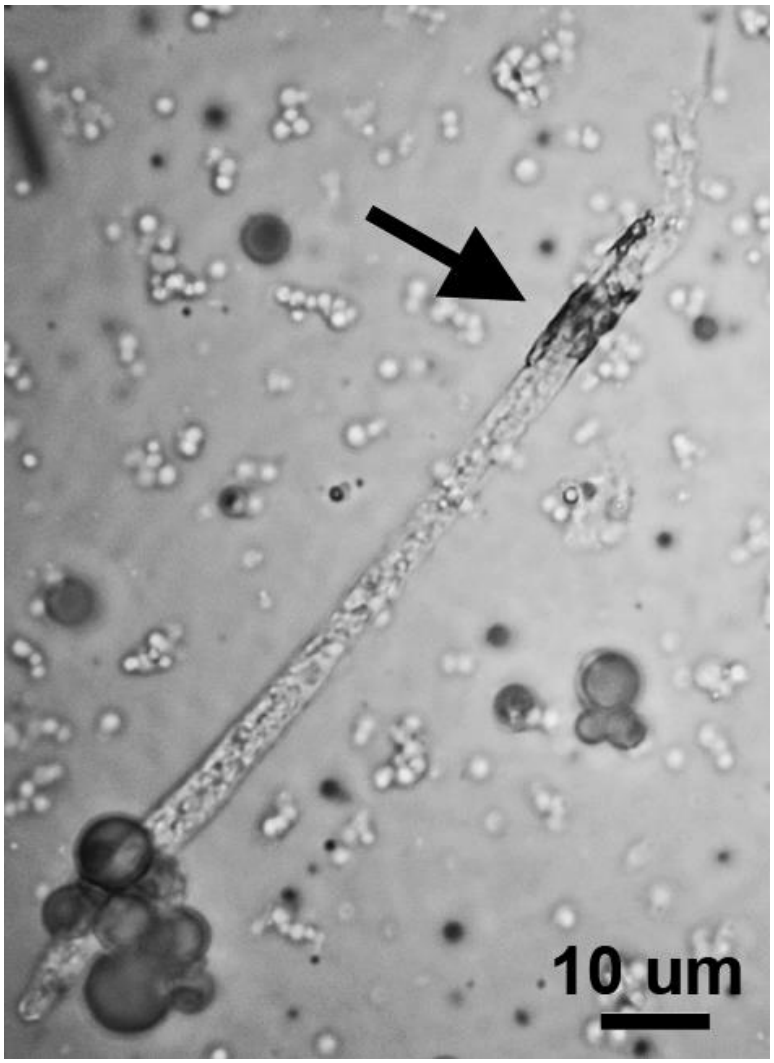

Figure 3.5 An example of a melanized microfilaria recovered from the hemocoel. LM showing melanization occurred on a part of the microfilaria body (arrow).